NASA TECHNICAL TRANSLATION NASA TT F-15926

BIOLOGICAL MOBILIZATION OF PHOSPHORUS AND NITROGEN IN INUNDATED SOILS USED FOR GROWING RICE

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Translation of "Biologicheskaya mobilizatsiya fosfora i azota v zatoplyayemykh pochvakh, ispol 'zuyemykh pod kulturu risa," Izvestiya Akademii Nauk KazSSR, Seriya Biologicheskaya, 8 (2), 19-28 (1970)

(NASA-TT-F-15926) BIOLOGICAL MOBILIZATION OF PHOSPHORUS AND NITROGEN IN INUNDATED SOILS USED FOR GROWING RICE (Kanner (Leo) Associates) 21 p HC $4.25 CSCL 08M G3/13

N74-32803

Unclas 48571

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
WASHINGTON, D.C. 20546 SEPTEMBER 1974

STANDARD TITLE PAGE

1. Report No. NASA TT F-15926	2. Government Accession No.	3. Recipient's Catalog No.
4. Title and Subtitle Biological Mobilization of Phosphorus and Nitrogen in Inundated Soils Used for Growing Rice		5. Report Date September 1974
		6. Performing Organization Code
7. Author(s) A. N. Ilyaletdinov, Institute of Microbiology and Virology, Academy of Sciences, Kazakh SSR		8. Performing Organization Report No.
		10. Work Unit No.
9. Performing Organization Name and Address Leo Kanner Associates Redwood City, CA 94063		11. Contract or Grant No. NASw-2481
		13. Type of Report and Period Covered Translation
12. Sponsoring Agency Name and Address National Aeronautics and Space Administration, Washington, D.C. 20546		14. Sponsoring Agency Code

15. Supplementary Notes

Translation of "Biologicheskaya mobilizatsiya fosfora i azota v zatoplyayemykh pochvakh, ispol 'zuyemykh pod kulturu risa," Izvestiya Akademii Nauk KazSSR, Seriya Biologicheskaya, 8 (2), 19-28 (1970)

16. Abstract

A direct correlation between accumulation of acid-soluble phosphorus and iron, hydrogen sulfide and sulfate-reducing bacteria in the soil was found. It was found that oxidation activities of microorganisms favor reduction in solution of iron and conversion of phosphorus to the free forms, in swampy meadow carbonate soils in a district of Kazakhstan. Tests using organic matter, in the form of alfalfa roots, in moderately wetted and inundated soil demonstrated a high microorganism population and low ammonia and easily hydrolyzed nitrogen content in moderately wetted soil, as well as suppression of microorganism growth and continually increasing content of available forms of nitrogen in inundated soils. Low ammonia concentration is likely due to assimilation of ammonia by microbes, nitrification and fixation of soil ammonia. Further tests were run on various combinations of soil, nitrogen, alfalfa root and mineral phosphate, on inundation of soil at various times before sowing rice.

17. Key Words (Selected by Author(s))		18. Distribution Statement Unclassified-Unlimited	
19. Security Classif. (of this report) Unclassified	20. Security Classif. (of this page) Unclassified	21. No. of Pages 19	22. Price

NASA-HQ

BIOLOGICAL MOBILIZATION OF PHOSPHORUS AND NITROGEN IN INUNDATED SOILS USED FOR GROWING RICE

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Nitrogen undergoes various conversions in the soil, in which /19*
biological factors, especially the activities of soil microorganisms, play an active role. Since plants better assimilate the most mobile nitrogen and phosphorus compounds, conversions connected with increase in availability of nutrient elements are of decisive importance. It is customary to call these processes mobilization.

A large part of the phosphorus in the soil and fertilizers introduced occur in the form of mineral phosphates. According to modern conceptions, the principal mobilizing factor, with respect to mineral compounds, mineral phosphates in particular, are acids, formed in the process of oxidation of organic matter by soil microflora. Meanwhile, an increase in mobility of mineral phosphate is observed in carbonate soils, which are subject to inundation. As is well known, no appreciable acid formation takes place upon inundation of carbonate soils; on the other hand, a considerable increase in alkalinity is noted.

The bulk of the soil nitrogen, in distinction from phosphorus, is organic compounds, which are unavailable for plant nutrition. Consequently, mobilization of it in the soil is continually connected with mineralization of organic compounds. An important source of available nitrogen in the soil can be the release of ammonia, fixed in clay minerals.

Investigation of the processes of mobilization of phosphorus and
nitrogen in the inundated swampy meadow carbonate soils of the /20

*Numbers in the margin indicate pagination in the foreign text

Kzyl-Ordinsk district is of interest, in connection with the cultivation of rice in these soils, the average yield of which in Kazakhstan, particularly in the Kzyl-Ordinsk district, is 30 centner/hectare at the present time. This is far from the limit for such a crop. The task of the investigators consists of finding ways of increasing the rice harvest, by means of efficient use of the potential fertility of the soil and increasing the effectiveness of fertilizer. This problem can be solved, on the basis of knowledge of the characteristics of the mobilization processes in inundated soil. The results of study of the patterns of mobilization of nutrients in inundated soil used for rice cultivation are correlated in this work. The studies were performed by colleagues of the Laboratory of General Microbiology, Institute of Microbiology and Virology, Academy of Sciences, Kazak SSR, Sh. Z. Mamilov and A. Adiyev, and colleague of Laboratory of Plant Physiology, Institute of Botany, Academy of Sciences, Kazak SSR, L. G. Krapivenko, under the general supervision of the author.

Connection between Phosphate Mobilization and Reduction of Sulfates and Iron by Microorganisms

The rate of reduction processes in inundated soil depends on microbiological activity, and they depend on the supply of organic matter in the soil, which serves as energy sources for their activities.

Considerable increase in group II phosphates (according to Chirkova), soluble in 0.5 N acetic acid, always is noted in inundated soil. Mandal (1964) observed an increase in the amount of phosphates in 0.5 N acetic acid in inundated soil, with decomposition of starch by spontaneous microflora. The cause of mobilization of mineral phosphates in soil under rice cultivation, in presence of decomposing alfalfa roots, the principal source of organic matter replenishment, should apparently be connected with processes which are most characteristic of inundated soils. Obviously, these are the processes of microbiological reduction of iron and sulfates.

In order to test the effect of the sulfate-reduction and biological reduction of iron on the dynamics of free phosphates, the following test was performed (Mamilov, Ilyaletdinov, 1969). Samples of swampy meadow carbonate soil were placed in test tubes (a portion of the sample was mixed with phosphorite). Then, the soil was flooded with a nutrient solution, containing 2 g/l ammonium sulfate and 5 g/l calcium lactate. The ammonium sulfate was used as the source of nitrogen and sulfates and the calcium lactate, as the source of organic matter, not containing phosphorus, for the microorganisms. Soil mixed with phosphorite, but submerged in distilled water, served as the control. All test tubes were set in a thermostat at a temperature of 30°. Observation of the sulfate-reducing microorganism populations, by the dynamics of hydrogen sulfide, free phosphates and iron content, was conductedfor a period of a month, at one-week intervals (Table 1).

The observation results demonstrated that introduction of a nutrient solution is favorable for the activity of sulfate-reducing bacteria. Vigorous growth of this group of bacteria leads to /21 vigorous reduction of sulfates and to accumulation of hydrogen sulfide.

The reduction process in inundated soil develops in such a manner, that reduction of trivalent ferric oxide to the divalent ferrous form takes place simultaneously or somewhat earlier than reduction of the sulfates. Divalent iron reacts easily with hydrogen sulfide; iron sulfide forms in this case. Spots of an intense black color, forming in inundated soil, are bound to precisely this compound. Iron sulfide is insoluble in water, but it dissolves well in weak acids. It is convenient to observe the dynamics of the content of this compound in the same 0.5 N acetic acid extract, which includes the group II phosphates (according to Chirkova.) A considerable amount of free iron is formed in tests with addition of nutrient solution containing organic matter.

TABLE 1

Change in content of free phosphates, iron and hydrogen sulfide and population of sulfate-reducing bacteria in water-flooded swampy meadow carbonate soil

Test Version	Day of incubation				
	Initial	7	14	21	28
Sulfate-reducing bacteria, 1000/g soil					
Soil + phosphorite + Ca lactate + $(NH_4)_2SO_4$	0.3	6.5	12.5	11.7	11.5
Soil + calcium lactate + $(NH_4)_2SO_4$	0.3	8.2	1.5	0.8	1.3
Soil + phosphorite	0.3	0.2	1.0	1.0	0.9
H_2S mg/100g soil					
Soil + phosphorite + Ca lactate + $(NH_4)_2SO_4$	0.0	7.8	9.0	7.2	8.6
Soil + calcium lactate + $(NH_4)_2SO_4$	0.0	8.4	7.7	8.8	7.8
Soil + phosphorite	0.0	1.1	1.1	1.0	1.3
Fe mg/100g soil					
Soil + phosphorite + Ca lactate + $(NH_4)_2SO_4$	0.6	12.2	16.6	17.6	16.6
Soil + calcium lactate + $(NH_4)_2SO_4$	0.6	10.2	16.6	16.6	15.6
Soil + phosphorite	0.6	6.0	8.0	9.6	9.6
P_2O_5 mg/100g soil					
Soil + phosphorite + Ca lactate + $(NH_4)_2SO_4$	1.2	5.5	8.4	8.6	8.6
Soil + calcium lactate + $(NH_4)_2SO_4$	0.8	2.7	6.3	6.2	6.1
Soil + phosphorite	1.2	3.8	2.4	2.2	2.4

Observations of the phosphate dynamics show that, in those tests in which favorable conditions for sulfate-reduction and biological reduction of iron were created, an appreciable increase in their availability occurs. Accumulation of a large amount of free phosphates, in tests with Aktyubinsk phosphorite, is evidence that mobilization of both soil and Aktyubinsk phosphorite phosphates take place.

Thus, the directly proportional relationship between accumu- /22 lation of phosphorus and iron in the soil, entering the 0.5 N acetic acid extract, hydrogen sulfide and sulfate-reducing bacteria population is successfully followed. All of these processes are closely connected with oxidation of organic matter by microorganisms.

S. P. Yarkov and colleagues (1950) determined the causative connections between formation of ferrous oxide and increase in solubility of phosphates in pozolic sod soils. They confirm that, with increase in amount of ferrous oxide, the solubility of phosphates is increased, which is connected principally with change of the slightly soluble ferric phosphates into the more soluble ferrous phosphates, with the setting free of part of the phosphoric acid by anaerobic decomposition of organic soil matter and with an increase in availability of calcium and magnesium phosphates for dissolution, as a consequence of removal of the protective films from the surfaces of the soil particles.

The formation of iron salt film on the surfaces of soil particles is not an unusual phenomenon. B. B. Polynov (1956) observed this process in nature, attributing great importance to the change of ferrous compounds into ferric. Silica participates, together with ferric oxide, in formation of film on the particle surfaces (Miller, 1967). McKeague and Cline (1963, 1963a) assert that at least part of the amorphous silica is encountered in the soil, in the form of films on the surface of ferric oxide and other compounds.

In order to increase the phosphoric acid availability, the iron-silica films, interfering with solution of these particles, must be removed from the surfaces of the phosphate particles. Silica becomes more soluble under alkaline conditions (Krauskopf, 1956; McKeague and Cline, 1963a), which are created in inundation of soils as a result of bacterial reduction of the sulfates (Ablakov, 1957; Ponomareva, 1962; Abd-el-Malek and Rizk, 1963).

Simultaneously with sulfate reduction under anaerobic conditions in the presence of decaying organic matter, biological reduction of iron to the soluble ferrous form takes place, as a result of which ferric oxides are removed from the surfaces of the soil particles. In this manner, in the presence of microorganisms oxidizing organic matter, favorable conditions are created in inundated soil, for solution of the protective films on the surfaces of soil minerals, phosphates in particular, a consequence of which is an increase in phosphoric acid availability. Removal of iron from solution, in the form of its sulfide, after reaction with hydrogen sulfide, also is a factor which facilitates phosphorus mobilization.

Accumulation of Free Nitrogen in Inundated Soil

In the soils of South Kazakhstan, the principal source of replenishment of organic matter is alfalfa root residues. Their decomposition provides agricultural crops with nitrogen.

We consider it interesting to follow how deamination of alfalfa root proteins with release of ammonia occurs, under conditions of moderate soil moisture (45% of complete wetness) and of inundation.

Two hundred fifty mg of finely cut alfalfa roots in capron bags/23 was placed in the soil and, periodically (after 15, 30 and 45 days), the amount of nitrogen in the decomposing vegetable material was accounted for. In decomposition of organic residues, under conditions of moderate wetting, vigorous growth of the microorganisms is observed. A low population of them is noted in inundated soil (Table 2). Anaerobiosis causes a deficit of oxygen in the soil, which contributes to massive death of aerobic microflora (Ilyaletdinov, Krapivenko, Mamilov, Diarova, Adiyev, 1969).

TABLE 2
Dynamics of microorganism population in decomposition of alfalfa roots, million per hectare of dry roots

Conditions of vegetable material decomposition in soil	Day of incubation		
	15	30	45
Ammonia-fixing bacteria			
Moderate wetting	10.9	94.0	246.0
Inundation	3.6	6.0	4.0
Fungi and bacteria assimilating mineral nitrogen			
Moderate wetting	16.1	214.0	794.0
Inundation	9.2	14.0	4.0

Under conditions of moderate wetting, the rate of ammonia fixation of vegetable proteins, expressed as the decrease in amount of nitrogen in the root residues, turned out to be low. In inundated soil, with comparatively weak growth of the microorganisms, a vigorous alfalfa protein deamination process took place. In half a month, the amount of nitrogen in the vegetable material decreased from 246 to 155 mg per 100 g of dry roots with moderate wetting and to 43 mg, with inundation, by 37 and 82.6%, respectively.

The fact that, despite the suppression of microorganism activity, vigorous proteolysis takes place in inundated soil, apparently is connected with activation of proteolytic enzymes in the cells of the alfalfa root residues. A burst of enzyme activity takes place at first, upon introduction of roots of it into the soil.

With moderate wetting, when the alfalfa residues are decomposing, two processes take place in the soil simultaneously: Ammonia fixation, accompanied by decomposition of the proteins and accumulation of ammonia, and nitrification, leading to formation of nitrates, by means of oxidation of ammonia by microorganisms.

Since nitrates are easily lost from inundated soil, as a result of washing out or bacterial reduction, accumulation of ammonia nitrogen in it is desirable in rice cultivation.

Suppression of nitrification can be achieved by replacement of the aerobic conditions by anaerobic in inundated soil. The comparative characteristics of accumulation of ammonia in inundated and uninundated soil, with ammonia fixation of alfalfa proteins, is presented in Table 3 (Ilyaletdinov, Mamilov, 1969).

TABLE 3
Dynamics of ammonia content, mg/100 g dry soil

Soil	Day of incubation				
	Initial	10	20	30	45
Inundated	2.14	2.27	4.35	4.09	4.47
Uninundated	2.14	1.84	3.48	3.23	--

More ammonia is accumulated unchanged in soil with inundation than with moderate wetting.

In the next series of tests, the moisture was maintained at the level of 50% of the total moisture capacity in the initial period of decomposition of alfalfa residues in the soil. At 10-day periods after the start of decomposition the soil was flooded with water, anaerobic conditions were created, and observations of the change in amount of ammonia in the soil were made (Table 4).

TABLE 4

Dynamics of ammonia accumulation vs. inundation times, mg/100 g dry soil

Day of incubation soil was inundated	Day of incubation				
	Initial	10	20	32	45
Without Inundation	2.23	--	2.29	1.20	1.16
10	2.23	2.67	2.63	2.66	2.75
20	2.23	2.06	2.95	1.97	1.94
30	2.23	2.06	--	1.53	1.56
40	2.23	--	2.33	1.20	1.28

The greatest amount of ammonia was accumulated with earlier (up to 10-20 days) inundation of the soil. With late inundation (30-40 days), its content differed little from the amount of ammonia in the control, uninundated soil. The section of Table 4 outlined with heavy lines corresponds to the water-flooding tests. /24

Thus, by means of decreasing the break in time between plowing the alfalfa and inundating the soil, a considerable amount of the nitrogen required by the plants can be preserved.

The ammonia freed, owing to deamination of the alfalfa proteins, is detected in the soil in the form of free ions. A series of experiments convinced us that a sharp increase in NH_4^+ content takes place in the soil with inundation, and that more ammonia is accumulated than with moderate wetting.

One of the indicators of availability of soil nitrogen is the content of easily hydrolyzed nitrogen, i.e., those forms of it which go into a weakly acid extract. As is well known, mineral and organic nitrogen compounds make up this fraction.

If the soil is treated with a weak acid after the mineral forms of nitrogen have been extracted, it is evident that the acid hydrolysate will be primarily organic nitrogen compounds.

We observed the dynamics of such compounds in composted soil, under aerobic and anaerobic conditions, with addition of 2% alfalfa roots. Under aerobic conditions, the size of the easily hydrolyzed nitrogen fraction fluctuates within narrow limits (from 5.9 to 6.7 mg per 100 g of soil). /25

By the end of composting, the content of easily hydrolyzed organic nitrogen (6.0 mg) changed little from the initial content (6.1 mg). In inundated soil, a gradual increase in amount of easily hydrolyzed organic nitrogen takes place. By the end of composting, the content of it reached 9.8 mg (Mamilov, 1969).

Still another proof of the favorable effect of biological and physicochemical processes on increase in ammonia availability is the observation of the dynamics of total and free ammonia in inundated soil, with addition of calcium lactate, which was introduced as a carbon compound, activating the microbiological processes and, at the same time, not changing the nitrogen balance of the soil.

In this case, the dynamics of the ammonia content depend on conversion of the ammonium cation, fixed in the mineral and organic portions of the soil. For extraction of NH_4^+, the method of Barshad was used, distillation with NaOH and KOH solutions. By heating soil with NaOH solution, all of the ammonia in the soil is released, and only free ammonia goes over in the distillate upon heating with KOH. Soil composting under anaerobic conditions leads to an increase in the total and, especially, the free ammonia, as a result of which the content of both forms of NH_4^+ becomes almost identical after 20 days, i.e., all the ammonia changes to the free form.

Under aerobic conditions, after a certain decrease at the start of incubation, an increase in amount of total ammonia occurs, but, simultaneously with this, a tendency towards decrease in its availability is noted.

In comparing the results of our tests of the number of microorganisms and dynamics of different forms of nitrogen, attention is drawn to the noncorrespondence between the high microorganism population and the low content of ammonia and easily hydrolyzed nitrogen in moderately wet soil. At the same time, the growth of microorganisms is suppressed in inundated soil, but the content of free forms of nitrogen, on the other hand, continually increases.

We think that the low ammonia concentration in moderately /26
wetted soil may be due to the following causes: a) Assimilation of ammonia by the microbe population; b) nitrification; c) ammonia fixation in the soil.

Decomposition of organic matter in wet soil, with good aeration, leads to vigorous growth of microflora. This abundantly producing microflora requires available sources of nitrogen, and it binds part of the ammonium nitrogen, released by decomposition of the alfalfa residues and soil humus, in the form of microbe plasma proteins.

Some idea of the importance of nitrification for decreasing the amount of ammonia can be obtained from familiarity with the results of observations of the dynamics of NH_4^+ and NO_3^- in soil mixed with alfalfa. Under aerobic conditions, the amount of NH_4^+ begins to decrease after three weeks of composting; a burst of nitrate formation is observed in this same period. There is every basis for thinking that oxidation of ammonia by nitrifying bacteria is taking place.

Under anaerobic conditions, nitrification does not take place, and existing nitrates disappear in the first week after inundation of the soil.

Effect of Flooding before Seeding on Rice Productivity

Inundating soil with water sharply changes the course of many physicochemical and biological processes, which cause conversion of nutrients. Better conditions for breakdown of vegetable proteins and organic matter by microorganisms, with release of the ammonium ion, the free form of nitrogen nutrition of rice, are created than in uninundated soil. The next element, which is important for plants, phosphorus, also changes to the free form by the action of microorganisms.

According to the observations of B. A. Neunylov (1962), in soils of the Primor'ye, increase in amount of ammonium nitrogen, owing to ammonification of vegetable proteins, takes place at slow rates in the spring and first half of the summer; as a result of this, the young rice plants frequently do not receive sufficient nutrition. In August, when the consumption of nitrogen decreases, it accumulates in the soil in excessive quantities.

In the soils of Kzyl-Ordinsk district, used for rice cultivation, the amount of ammonia increases, depending on their saturation with organic matter, for a period of 1.5-2 months after inundation (Chirkova, 1960), when nitrogen and phosphorus consumption by the plants decreases considerably. Consequently, as a result of inundation of the plots simultaneously with rice sowing, ammonia and phosphoric acid accumulate slightly in the soil, and the seedlings, not supplied with nitrogen and phosphorus in the early growth period, are deprived of the possibility of producing high yields.

In practice, the disparities in time between the dynamics of the naturally occurring processes of ammonification and phosphate mobilization in the soil and the nitrogen and phosphorus requirements of the rice are eliminated by application of mineral fertilizers at the start of growing. In this manner, the assumption in the practice of agricultural technology of rice sowing, according to which sowing and soil inundation are carried out at the same time, does not permit efficient use of the potential fertility of the soil.

In order to test the correctness of the consideration stated, we carried out a test, growing rice in soil in growing containers, with inundation at different times, in the following versions: a) soil + nitrogen; b) soil + alfalfa roots + nitrogen; c) soil /27 + alfalfa roots + phosphorite flour + nitrogen. The alfalfa roots (40 g) and phosphorite flour from the Aktyubinsk deposit (2.8 g) were introduced into containers containing 9 kg of dry soil when filled. This amount of root residues corresponds approximately to the content of them in alfalfa beds, and the phosphorite phosphorus to 90 kg/hectare P_2O_5. Ammonium sulfate (2.4 g per container) was introduced with water in phase 1 of leafing. This amount is equal to 120 kg/hectare of fertilizer.

The soil was flooded 32, 22, 12 and 7 days before sowing.

The principal indicators of productivity of the rice plant is grain yield (Table 5).

TABLE 5
Grain yield and weight of growing mass of rice plant vs. presowing inundation, g/100 plants

Test	Soil inundation before sowing by							
	7 da	12 da	22 da	32 da	7 da	12 da	22 da	32 da
	Grain				Straw			
N_{120} (Control)	128 ±2.3	129 ±1.0	190 ±1.7	230 ±0.9	120	129	200	240
N_{120} + (Alfalfa)	230 ±2.0	250 ±3.6	280 ±0.2	295 ±5.3	230	250	320	370
N_{120} P_{90} (Alfalfa and Aktyubinsk phosphorite)	183 ±1.7	240 ±1.4	290 ±2.0	308 ±3.0	230	241	280	330

By inundating the soil shortly before sowing (7 days), the rice grain yield was 128 g per 100 plants in the control test, 230 g in containers into which ammonium sulfate and alfalfa root residues were introduced and, where ammonium sulfate, roots and an insoluble source of phosphorus, phosphorite, was added to the soil, 183 g. The weight of grain in the tests exceeded the amount of it in the control by 80 and 43%.

By inundating the soil 12 days before sowing, the grain yield in the control was unchanged from that with inundation 7 days before sowing. With introduction of alfalfa root, the yield increased negligibly, from 230 to 250 g per 100 plants and, on a background of alfalfa roots with phosphorite flour, from 183 to 240 g. The excess of grain weight in the tests over that in the controls was 94 and 80%, respectively.

With soil inundation 22 days before sowing, the rice yield in the control increased to 190, compared with 129 g with 12-day inundation. The weight of grain in the tests increased. Thus, with addition of alfalfa, the grain harvest increased from 250 to 280 g and, alfalfa with phosphorite, from 240 to 290 g per 100 plants. The yield in the tests exceeded the control by 47 and 53%.

By flooding the soil 32 days before sowing, the maximum yield was obtained in all test versions. In the control, without use of fertilizer, the grain weight was 230 g, i.e., the same as in inundation 7 days before sowing, with introduction of nitrogen, and almost twice that in the control, in which the soil was inundated 7 days before sowing. In the test using nitrogen, the grain weight was 194 g and, nitrogen and Aktyubinsk phosphorite on an alfalfa background, 308 g.

Attention is deserved to the fact that earlier presowing inundation of the soil has a favorable effect on yield on a poor background of fresh organic matter. Organic matter of such soils was in forms which are difficult to decompose.

In inundating 32 days before sowing, the grain weight was twice that with inundation 0-12 days beforehand, and it approximated the level in containers, into which alfalfa root residues were introduced. Soils enriched with fresh organic matter provided a high absolute yield of grain, relative to the poor background. The difference in weight obtained between tests with soil inundation 32 and 7 days before sowing was 17-30% (Ilyaletdinov, Krapivenko, Mamilov, 1969).

We note again in conclusion that the best growth and yield of rice after preliminary (20-30 days before sowing) inundation of the soil provided a better supply to the plants of nitrogen and phosphorus, changed to available forms as a result of the activities of microorganisms.

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